antler, and an illustration was given, but the rodlets were not clear. In this study, rodlets could be photographed clearly. The rodlets were observed running around not 100% but about 85% of the gonotyl circumference radially, and this observation agrees with that reported by Chai et al. (1986). The present study revealed that the rodlets are not a single rod but are composed of 3 to 6 spines in a row appearing as "cockscomb."

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Literature Cited

Adams, K. O., D. L. Jungkind, E. J. Bergquist, and C. W. Wirts. 1986. Intestinal fluke infection as a result of eating sushi. American Journal of Clinical Pathology 86:688–689.

Beaver, P. C., R. C. Jung, and E. W. Cupp. 1984. Clinical Parasitology, 9th ed. Lea & Febiger, Philadelphia, Pennsylvania. Pp. 478–480.

Chai, J. Y., H. L. Chung, M. H. Choi, W. M. Sohn, S. J. Hong, and S. H. Lee. 1992. Surface ultrastructure of *Heterophyes nocens* (Trematoda: Heterophyidae). Korean Journal of Parasitology 30: 75–82.

———, B. S. Seo, S. H. Lee., S. J. Hong, and W. M. Sohn. 1986. Human infections by *Heterophyes heterophyes* and *H. dispar* imported from Saudi Arabia. Korean Journal of Parasitology 24:82–88.

Fujino, T., H. Higo, Y. Ishii, S. Saito, and E. R. Chen. 1989. Comparative studies on two similar species of *Haplorchis* and *Metagonimus* (Trematoda: Heterophyidae)—surface ultrastructure of adults and eggs. Proceedings of the Helminthological Society of Washington 56:35–41.

Hong, S. J., J. Y. Chai, and S. H. Lee. 1991. Surface ultrastructure of the developmental stages of *Heterophyopsis continua* (Trematoda: Heterophyidae). Journal of Parasitology 77:613–620.

Kagei, N., S. Hayashi, and K. Kato. 1980. On the heterophyid trematoda (*Heterophyes heterophyes*) infection cases imported from Egypt to Japan. Japanese Journal of Tropical Medicine and Hygiene 8:1–7. (In Japanese with English abstract.)

Miyazaki, I., and Y. Toh. 1988. Parasitic Zoonoses. Kyushu University Press, Fukuoka. Pp. 219–234. (In Japanese.)

Saito, S. 1972. On the differences between *Metagonimus yokogawai* and *Metagonimus takahasii*. 1. The morphological comparisons. Japanese Journal of Parasitology 21:449–458. (In Japanese with English abstract.)

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Research Note

Applicability of Crude Extracts of Adult *Spirometra erinacei* for Serodiagnosis of Sparganosis

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ABSTRACT: Antigenicity of crude extracts of adult *Spirometra erinacei* was evaluated in comparison to those of the plerocercoid (sparganum) for serodiagnosis of human sparganosis. Patients' sera from 39 sparganosis, 77 other helminthic diseases, and 50 uninfected controls were tested by enzyme-linked immunosorbent assay (ELISA). When both extracts were used as antigen, specific antibody levels in sparganosis sera were highly correlated (r = 0.83). The sensitivity and specificity of the adult worm extracts were 92.3 and 98.3%, while those of the sparganum were 94.3 and 96.6%, respectively. This result showed that crude extracts of adult *S. erinacei* could be used as a diagnostic antigen of sparganosis.

KEY WORDS: *Spirometra erinacei*, sparganum, sparganosis, antigen, immunodiagnosis.

Human sparganosis is a parasitic disease caused by tissue-invading plerocercoids of *Spirometra* spp. (sparganum) such as *S. erinacei* Faust, Campbell, and Kellogg 1929 or *S. mansonoides* (Mueller 1935) Wardle, McLeod and

Stewart 1947. The parasites usually infect the subcutaneous and muscle layer but may invade the visceral organs and the central nervous system, sometimes eliciting severe neurological manifestations (Chang et al., 1992; Moon et al., 1993). The infection has been reported worldwide, but most cases were detected in China, Korea, Japan, and Southeast Asia (Holodniy et al., 1991).

In serological diagnosis of the disease, the crude extracts of the sparganum have been used as an antigen (Kim et al., 1984; Nishyama et al., 1994). Unlike in the past, however, it is getting difficult to obtain the worms from naturally infected hosts such as snakes or frogs due to the decrease of their population and degradation of the environment by industrialization. Unless the sparganum can be supplied in sufficient quantities from laboratory maintenance, we should search for another source of antigen for the serological diagnosis. In this respect, the adult worm may be a source if its antigenicity is shown to be useful. We evaluated here the applicability of crude extracts of adult *S. erinacei* as a diagnostic antigen.

Spargana, collected from snakes, *Rhabdophis tigrinus tigrinus* Boie, 1826, were washed with physiological saline and ground with a teflon-pestle tissue homogenizer followed by centrifugation at 20,000 *g* for 1 hr. The resulting supernatant was regarded as the crude sparganum extracts (Kong et al., 1994b). A dog was infected with 2 spargana *per os*. Two months later, adult strobila, which were confirmed under the dissecting microscope (wet weight: 8.2 g), were recovered from the dog's intestine. The strobila were washed and homogenized using a Waring blender 8 times at 20,000 rpm for a total of 15 min. Supernatant was obtained as described above. All procedures were carried out at 4°C. Protein content of the sparganum extracts was 6.4 mg/mL and that of adult extracts, 5.5 mg/mL, by the method of Lowry et al. (1951).

Sera from a total of 39 sparganosis, 24 cysticercosis, 5 each of *Taenia saginata* and *Diphyllobothrium latum* infections, 23 clonorchiasis, 20 paragonimiasis, and 50 uninfected controls without any possible infection were used. They were diagnosed either by positive antibody reaction (28 cases of sparganosis), or surgically (11 cases of sparganosis), or by egg detection (all other patients).

Using the crude extracts of spargana and adult

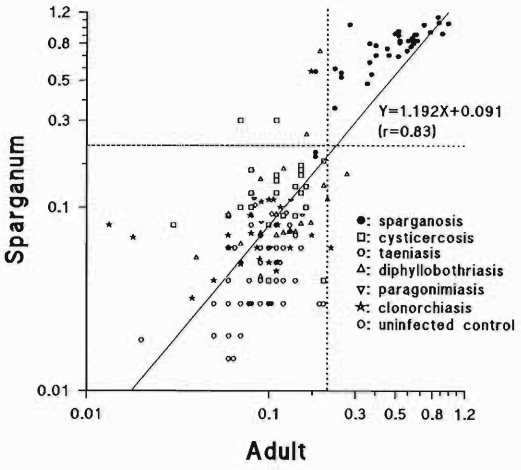

Figure 1. Correlation between antibody levels measured by ELISA absorbance using the adult and sparganum extracts as antigen. Sera from 39 sparganosis, 77 other helminthic diseases, and 50 normal controls were examined. Positive criteria at abs. 0.22 are indicated by dotted lines.

S. erinacei as antigens, antisparganum specific antibody (IgG) levels in the sera were measured. Antigen (200 μL), diluted to 2.5 μg/mL in carbonate buffer (0.05 M, pH 9.6), was coated to a microtiter plate (Costar, Cambridge, MA) overnight at 4°C. The sera (200 μL) were diluted to 1:100 in phosphate-buffered saline containing 0.05% Tween 20 (PBS/T, pH 7.4) and reacted for 2 hr at 37°C. Peroxidase-conjugated antihuman IgG (heavy- and light-chain specific, Cappel, Durham, NC) was diluted to 1:1,000 in PBS/T and further incubated for 2 hr at 37°C. The color reaction was developed using *o*-phenylenediamine and stopped by adding 25 μL of 8 N H_2SO_4. Absorbance was read at 490 nm using an ELISA reader (Bio-Rad M 3550, Bio-Rad, Hercules, CA). Cut-off absorbance for a positive reaction was set at 0.22 for both antigens (Kim et al., 1984).

Figure 1 shows the correlation of antibody levels as expressed as absorbance (abs.) in the sera tested. Mean abs. in sera of 39 sparganosis patients was 0.51 (±0.21) for the adult extracts, while that of the sparganum was 0.76 (±0.22) (Table 1). Out of 39 sparganosis sera, 36 cases (92.3%) exhibited positive reactions to the adult antigen, whereas 37 sera (94.3%) were positive to the sparganum antigen. Sera of 2 paragonimiasis, 1 clonorchiasis, and 2 cysticercosis cases exhibited a positive reaction to the sparganum

Table 1. Mean ELISA absorbance against each antigen preparation.

Disease category	No. of sera examined	Mean absorbance ± standard deviation	
		Adult	Sparganum
Sparganosis	39	0.51 ± 0.21	0.76 ± 0.22
Cysticercosis	24	0.12 ± 0.05	0.12 ± 0.04
Taeniasis	5	0.11 ± 0.04	0.13 ± 0.04
Diphyllobothriasis	5	0.11 ± 0.06	0.10 ± 0.05
Paragonimiasis	20	0.13 ± 0.08	0.13 ± 0.14
Clonorchiasis	23	0.10 ± 0.05	0.09 ± 0.09
Uninfected control	50	0.09 ± 0.04	0.04 ± 0.02

extracts (specificity: 96.6%). Meanwhile, 2 paragonimiasis and 1 clonorchiasis serum showed a positive reaction to the *S. erinacei* extracts (specificity: 98.3%). The regression equation of the abs. was $Y = 1.192X + 0.091$ (Y = absorbance for sparganum extracts, X = absorbance for *S. erinacei* extracts) ($r = 0.83$, $P < 0.01$). Antibody levels in other helminthic diseases and uninfected controls are summarized in Table 1.

Diagnosis of human sparganosis largely depends on the identification of the worms recovered from excisional biopsy. With progress in imaging diagnosis, however, preoperative presumption is now possible when the larva invades the central nervous system (Chang et al., 1992; Moon et al., 1993). Ultrasonographic findings of an elongated, folded bandlike hypoechoic structure in a heterogeneous hyperechoic mass are strongly suggestive of subcutaneous sparganosis (Chung et al., 1995). In addition, when unidentified worm sections were revealed in the pathological specimens, a specific antibody test is supplementary to confirm or disregard the presumption. The antibody test is also a useful seroepidemiological survey tool (Kong et al., 1994a).

The present study showed that the adult extracts of *S. erinacei* could be used in the serological diagnosis of sparganosis. Diagnostic sensitivity and specificity of both antigens were in 92–98%. The 2 sparganosis cases that did not react positively to either antigen were surgically confirmed chronic cerebral sparganosis in which highly degenerated worms were identified (Moon et al., 1993). These cases were included in the antigen evaluation to cover the spectrum of the disease.

In conclusion, while the adult worm extracts exhibited lower activity than those of the sparganum in capturing the specific IgG antibody in the patients' sera, they revealed similar sensitivity and specificity with the sparganum extracts. In addition, adult worms have the benefit of providing large amounts of antigen with easy manipulation.

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Literature Cited

Chang, K. H., J. G. Chi, S. Y. Cho, M. C. Han, D. H. Han, and M. C. Han. 1992. Cerebral sparganosis: analysis of 34 cases with emphasis on CT features. Neuroradiology 34:1–8.

Chung, K. H., K. S. Park, Y. Lee, and C. K. Park. 1995. Breast sparganosis: mammographic and ultrasound features. Journal of Clinical Ultrasound 23:447–451.

Holodniy, M., J. Almenoff, J. Loutit, and G. K. Seinberg. 1991. Cerebral sparganosis: case report and review. Review of Infectious Diseases 13:155–159.

Kim, H., S. I. Kim, and S. Y. Cho. 1984. Serologic diagnosis of human sparganosis by means of micro-ELISA. Korean Journal of Parasitology 22: 222–228.

Kong, Y., S. Y. Cho, and W. S. Kang. 1994a. Sparganum infections in normal adult population and epileptic patients in Korea: a seroepidemiological observation. Korean Journal of Parasitology 32: 85–92.

———, Y. B. Chung, S. Y. Cho, S. H. Choi, and S. Y. Kang. 1994b. Characterization of three neutral proteases of *Spirometra mansoni* plerocercoid. Parasitology 108:359–368.

Lowry, O. H., N. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement by Folin phenol reagent. Journal of Biological Chemistry 193:265–275.

Moon, W. K., K. H. Chang, S. Y. Cho, M. H. Han, S. H. Cha, J. G. Chi, and M. C. Han. 1993. Cerebral sparganosis: MR imaging versus CT features. Radiology 188:751–757.

Nishyama, T., T. Ide, S. R. Himes, and S. Ishzaka. 1994. Immunodiagnosis of human sparganosis mansoni by micro-chemiluminescence enzyme-linked immunosorbent assay. Transactions of the Royal Society of Tropical Medicine and Hygiene 88:663–665.